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भारतीय मानक

खाने योग्य मध्यम — वसा सोया आटा — विशिष्टि

(पहला पुनरीक्षण)

Indian Standard

EDIBLE MEDIUM — FAT SOYA FLOUR — SPECIFICATION

(First Revision)

ICS 67.060

B U R E A U O F I N D I A N S T A N D A R D S
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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Foodgrains, Starches and Ready-to-Eat Foods Sectional Committee, FAD 16

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Foodgrains, Starches and Ready-to-Eat Foods Sectional Committee had been approved by the Food and Agriculture Division Council.

India produces about 7.3 million tonne of soyabean annually. Soyabean is a grain legume and contains about 40 percent good quality protein, 30 percent carbohydrates, 20 percent fat and reasonable amounts of minerals and vitamins. Soyabean and soya products are nutritious, economical and health promoting. Soyabean protein is rich in lysine, an essential amino acid, and can therefore, serve as a nutritionally useful ingredient and supplement to cereal products in blended and processed foods. Soyabean products are manufactured in the country in many forms, such as full-fat, medium-fat and low-fat flours, extruded products and protein isolates, and there is a scope for developing others. However, soyabean requires careful processing to transform/make it fit for human consumption.

Separate Indian Standards have been formulated to cover the requirements of full-fat, medium-fat and low-fat edible soya flours and of soya protein isolates. The standard on medium-fat soya flour was first published in 1975. This standard is now being revised and the following major modifications from the earlier version have been incorporated:

a) Existing chemical and microbiological requirements have been made stricter;
b) Additional requirements like urease activity, trypsin inhibitor activity, free fatty acid content, absence of *Staphylococcus* have been included; and
c) Modifications in packaging and labelling requirements.

In the formulation of this standard, due consideration has been given to the provisions of *Food Safety and Standards Act*, 2006 and Rules framed thereunder and the *Legal Metrology (Packaged Commodities) Rules,* 2011. However, this standard is subject to restrictions imposed under these, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding-off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

EDIBLE MEDIUM — FAT SOYA FLOUR — SPECIFICATION

(First Revision)

1 SCOPE

This standard prescribes the requirements and methods of sampling and test for edible medium-fat soya flour.

2 REFERENCES

The standards given below contains provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of these standards:

IS No.	*Title*
323 : 2009	Rectified spirit for industrial use — Specification (*second revision*)
1070 : 1992	Reagent grade water — Specification (*third revision*)
2491 : 1998	Food hygiene — General principles — Code of practice (*second revision*)
2566 : 1993	Specification for B-twill jute bags for packing foodgrains (*third revision*)
4684 : 1975	Edible groundnut flour (expeller pressed) (*first revision*)
4876 : 1986	Specification for edible cottonseed flour (solvent extracted) (*first revision*)
5401 (Part 1) : 2012/ISO 4832 : 2006	Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms: Part 1 Colony count technique (*second revision*)
5402 : 2012/ ISO 4833 : 2003	Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of micro-organisms — Colony count technique at 30°C (*second revision*)
5403 : 1999	Method for yeast and mould count of food stuffs and animal feeds (*first revision*)
5887 (Part 3) : 1999/ ISO 6579 : 1993	Methods for detection of bacteria responsible for food poisoning General guidance on methods for detection of *Salmonella*
(Part 8/Sec 1) : 2002/ISO 6888-1 : 1999	Horizontal method for enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), Section 1 Technique using Baird-Parker agar medium
(Part 8/Sec 2) : 2002/ISO 6888-2 : 1999	Horizontal method for enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), Section 2 Technique using rabbit plasma fibrinogen agar medium
10171 : 1999	Guide on suitability of plastics for food packaging (*second revision*)
14818 : 2000/ ISO 13690 : 1999	Cereal and pulses and milled products — Sampling of static batches

3 REQUIREMENTS

3.1 Description

The medium-fat soya flour shall be prepared after partial extraction or expression of oil from cleaned, sound and wholesome soyabeans. It shall be in the form of coarse or fine powder, or grits; white or creamy-white to yellow in colour.

3.2 Edible medium-fat soya flour shall be free from rancid odour, extraneous matter, insects, insect fragments (living and dead), rodent hair, excreta and fungal infestation and other foreign matter. The product shall be free from any artificial colouring matter or flavouring agents.

3.3 Hygienic Conditions

The product shall be manufactured, packed and stored under strict hygienic conditions (*see* IS 2491).

3.4 The product shall also conform to the requirements given in Table 1.

4 PACKING AND MARKING

4.1 Packing

4.1.1 The material shall be packed in food grade plastic material (*see* IS 10171) or polyethylene-lined jute bags or in clean tin-plate containers conforming to IS 2566

or in any other food grade packing material as agreed to between the purchaser and supplier. If packed in jute bags, the mouth of each bag shall be either machine-stitched or hand stitched. If it is hand stitched, the mouth shall be rolled over and then stitched. The stitches shall be in two cross rows with at least 14 stitches in each row.

4.2 Marking

4.2.1 The following particulars shall be marked or labelled legibly and indelibly on each container:

a) Name of the material, and trade-mark, if any;
b) Name and address of the manufacturer;
c) Batch or code number;
d) Net quantity, in gram or kilogram;
e) Date of manufacture;
f) List of ingredients;
g) Nutritional contents such as protein, fat, carbohydrates, dietary fibre; minerals and vitamins;
h) Instructions as to how to use the product;
j) The words 'Best before.....', (the date to be given by the manufacturer); and
k) Any other information required under the *Legal Metrology (Packaged Commodities) Rules*, 2011 and *Food Safety and Standards Act*, 2006 and the Rules framed thereunder.

4.2.2 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

4.2.2.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act,* 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

5 SAMPLING

Representative samples of the material shall be drawn and conformity of the material to the requirements of the specification shall be determined according to the procedure given in IS 14818.

6 TESTS

6.1 Tests shall be carried out by the methods referred to in col 4 and col 5 of Table 1.

6.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

Table 1 Requirements for Edible Medium-Fat Soya Flour
(*Clause* 3.4)

Sl No.	Characteristics	Requirement	Method of Test, Ref to	
			Annex	Other Indian Standard
(1)	(2)	(3)	(4)	(5)
i)	Moisture, percent by mass, *Max*	8	—	Appendix B of IS 4684
ii)	Protein, percent by mass, *Min*	44	—	Appendix C of IS 4684
iii)	Crude fat, percent by mass, *Max*	5	—	Appendix F of IS 4684
iv)	Total ash (on dry basis), percent by mass, *Max*	6	—	Appendix D of IS 4684
v)	Acid insoluble ash (on dry basis), percent by mass, *Max*	0.3	—	Appendix E of IS 4684
vi)	Crude fibre (on dry basis), percent by mass, *Max*	3.5	—	Appendix H of IS 4684
vii)	Trypsin inhibitor activity, percent of the original, *Max*	75	A	—
viii)	Urease activity (change in *p*H unit)	Nil	B	—
ix)	Total free fatty acid, percent by mass, *Max*	0.99	C	—
x)	Available lysine, gram per six gram of nitrogen, *Min*	5.5	—	Appendix A of IS 4876
xi)	Total bacterial count, per gram, *Max*	50 000	—	IS 5402
xii)	Coliforms, per gram	Absent	—	IS 5401 (Part 1)
xiii)	*Staphylococcus aureus,* per 25 gram	Absent	—	IS 5887 (Part 8/Sec 1 and Sec 2)
xiv)	*Salmonella,* per 25 gram	Absent	—	IS 5887 (Part 3)
xv)	Yeast and mould count, per gram, *Max*	100	—	IS 5403
xvi)	Aflatoxin, g/kg, *Max*	30	—	Appendix J of IS 4684

ANNEX A

[*Table* 1, *Sl No.* (vii)]

DETERMINATION OF TRYPSIN INHIBITOR ACTIVITY

A-0 GENERAL

A-0.1 The method is applicable to the determination of the levels of trypsin inhibitor activity in whole soyabeans and in various soyabean products. Trypsin inhibitor activity is evaluated by measuring the extent to which the ability of trypsin to liberate *p*-nitroanilide with benzoyl arginyl para nitroanilide (BAPNA) solution is retarded by inhibitor present in soyabean and soya products. The reagent BAPNA solution is allowed to form a coloured complex with trypsin in the presence and absence of trypsin inhibitor for a specified period. The reaction is stopped and the colour is determined by measuring the absorption at 410 nm.

A-1 APPARATUS

A-1.1 Homogenizer or Blender or Mechanical Shaker, of suitable type.

A-1.2 Water-Bath, capable of maintaining temperature of 37 ± 0.5°C.

A-1.3 Centrifuge, provided with 20-25 ml round-bottom centrifuge tubes; 3 000 rpm.

A-1.4 Spectrophotometer, of suitable type.

A-2 REAGENTS

A-2.1 Trypsin Solution — Accurately weigh 0.002 g of trypsin and dilute to 100 ml with 0.001 N HCl. The solution may be stored for as long as one month at 5 - 10°C without appreciable loss in percent activity.

A-2.2 Tris Buffer — Weigh accurately 1.21 g of tris (hydroxymethyl) aminomethane and 0.59 g of $CaCl_2.2H_2O$ and dissolve in 180 ml of distilled water. Adjust the *p*H to 8.2 with 1 N HCl and make up the volume to 200 ml with distilled water.

A-2.3 BAPNA (Benzoyl Arginyl *p-a* Nitroanilide) Substrate — Weigh accurately 0.080 g of BAPNA and dissolve in 2 ml of dimethyl sulphoxide and dilute to 200 ml with pre-warmed tris-buffer. The solution is stable up to 4 h.

A-2.4 0.01 N Sodium Hydroxide Solution — Dissolve 0.4 g of sodium hydroxide in distilled water and make up the volume to 1 000 ml with distilled water.

A-2.5 0.001 M Hydrochloric Acid — Dilute 0.088 ml of concentrated hydrochloric acid to 100 ml with distilled water.

A-2.6 30 Percent Acetic Acid — Dilute 30 ml of acetic acid to 100 ml with distilled water.

A-3 PROCEDURE

A-3.1 Extracting Trypsin Inhibitor from Soya Products

Defatted soya meal is used for determination of trypsin inhibitor. When necessary, grind the soya product without generating excessive heat so that 60 percent passes through a 425 micron IS sieve (equivalent to No. 40 US Standard sieve). One gram of finely ground defatted sample is extracted with 50 ml of 0.01 N sodium hydroxide for 3 h on a shaker at room temperature (low setting). The *p*H of the resulting suspension should be within 8.4 to 10.0. This suspension is then diluted with distilled water to the point where 1.0 ml of the sample extract produces trypsin inhibition of 40-60 percent of the trypsin used as a standard in the analysis. The limitation is needed to reduce the relative standard deviation.

A-3.2 Determination of Trypsin Inhibitor Activity

The assay reaction consists of 0.5 ml of trypsin solution, 0.5 ml water and 1.25 ml of substrate (BAPNA solution). The reaction is carried out at 37°C for exactly 10 min and the reaction is arrested by adding 0.25 ml of 30 percent acetic acid. The absorbance of para nitroanilide liberated is measured at 410 nm against an appropriate blank in which the reaction is arrested by adding 30 percent acetic acid prior to the addition of BAPNA. The trypsin standard contains no soya samples.

A-4 CALCULATION

$$\text{Trypsin inhibitor activity, mg/g of sample} = \frac{(A_1 - A_2) \times D}{0.019 \times W_1 \times 1\,000 \times W_2}$$

where

A_1 = absorbance measured at 410 nm for trypsin standard blank containing no sample extract;

A_2 = absorbance measured at 410 nm for trypsin standard containing sample extract;

W_1 = weight of the sample, in g;

W_2 = amount of the sample taken, in ml; and

D = dilution factor which includes 50 of the original extraction with sodium hydroxide and further dilution with distilled water.

NOTES

1 TU is Trypsin unit. One trypsin unit is arbitrarily defined as an increase of 0.01 absorbance units at 410 nm under the condition of assay.

2 Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TUI).

ANNEX B

[*Table* 1, *Sl No.* (viii)]

DETERMINATION OF UREASE ACTIVITY

B-0 GENERAL

The method is of special applicability to the urease activity in soyabeans, products derived from them like full-fat, medium-fat and low-fat flours, extruded products and isolates. Loss of urease activity at high levels is sensitive to improvement in protein quality, but measurement of urease activity at low levels is a less sensitive index of protein quality.

B-1 APPARATUS

B-1.1 Water Bath, capable of maintaining temperature of 30 ± 0.5°C.

B-1.2 *p*H Meter, fitted with glass and calomel electrodes and equipped to test 5 ml of solutions.

B-1.3 Test Tubes, 20 mm × 150 mm, fitted with ground glass or rubber stoppers.

B-1.4 Volumetric Flask, 1 000 ml capacity.

B-2 REAGENTS

B-2.1 0.05 M Phosphate Buffer Solution — Dissolve 3.403 g of potassium dihydrogen phosphate ($KH_2PO_4.7H_2O$) in approximately 100 ml of freshly distilled water. Dissolve 4.355 g of dipotassium hydrogen phosphate ($K_2HPO_4.H_2O$) in approximately 100 ml of water. Combine the two solutions and make up the volume to 1000 ml. Before using, adjust the *p*H of the solution to 7.0 with either a solution of strong acid or a strong base. The buffered solution has a useful life of less than 3 months.

B-2.2 Buffered Urea Solution — Dissolve 15 g of urea in 500 ml of phosphate buffer solutions (*see* **B-2.1**). Add 5 ml of toluene to serve as a preservative and prevent mould formation. Adjust the *p*H of this urea solution to 7.0 using a solution of either a strong acid or a strong base.

B-3 PROCEDURE

B-3.1 Where necessary, grind the sample without generating excessive heat so that 60 percent passes through a 425 micron IS Sieve (equivalent to No. 40 US Standard sieve).

B-3.2 Weigh 0.200 g of the material into a test tube. Add 10 ml buffered urea solution, stopper and mix without inverting and place in the water bath at 30°C.

B-3.3 Place 1 g of the ground sample of the soya product in a small glass beaker or watch glass and heat it at 135°C for 30 min to inactivate the enzyme. Weigh 0.200 g of the inactivated material into a test tube. Add 10 ml of phosphate buffer, stopper and mix without inverting and place in the water bath at 30°C. It is convenient to place the test and blank test tubes in the water bath at exactly 5 min intervals using a stop watch.

B-3.4 Mix the contents of both the sample and the blank test tubes every 5 min during the digestion period. At the end of 30 min, remove each tube from the water bath, transfer the supernatant liquid to a 5 ml beaker and after exactly 5 min determine the *p*H.

B-4 CALCULATION

The difference between the *p*H of the test sample (higher) and that of the blank is a measure of the urease activity present in the sample.

NOTE — Fresh raw soyabeans, which give a *p*H change of always over 1 unit, may be usefully run as checks during urease activity determinations.

ANNEX C

[*Table* 1, *Sl No.* (ix)]

DETERMINATION OF FREE FATTY ACIDS

C-0 GENERAL

The acid value is determined by directly titrating the material in an alcoholic medium with aqueous sodium or potassium hydroxide solution. Free fatty acid is calculated as oleic, lauric, ricinoleic or palmitic acids. Since the acid value/free fatty acids (FFA) from the soya products like full-fat soya flour, medium-fat soya flour and low-fat soya flour are to be assayed, the oil/fat may be extracted prior to the determination using a standard procedure as given in **C-1**.

C-1 ETHER EXTRACT

C-1.1 The ether extract method measures the proportion of soya flour that is soluble in ether. It is

equivalent to the total amount of lipids present in soya flour and it represents mostly true fats and oils. However, it also includes some ether soluble compounds that are not true fats like fat soluble vitamins, carotenes, chlorophylls, sterols, phospholipids, waxes and cutins. Fatty acids shall readily form insoluble complexes with free cations, most notably calcium. The reaction may occur in soya flour that has a relatively high concentration of positively charged minerals. To assume that all the fat components are extracted from a mineral rich sample, it is recommended to perform an acid hydrolysis in hot hydrochloric acid prior to the ether extraction.

C-1.2 REAGENTS

C-1.2.1 *Hydrochloric Acid*, 3 N.

C-1.2.2 *Anhydrous Diethyl Ether*

C-1.3 APPARATUS

C-1.3.1 *Water Bath*

C-1.3.2 *Hot Air Oven*, capable of maintaining temperature of 105 ± 1°C.

C-1.3.3 *Soxhlet Extraction System*

C-1.3.4 *Filter Paper*

C-1.3.5 *Erlenmeyer Flask,* 250 ml capacity.

C-1.4 PROCEDURE

C-1.4.1 Weigh a suitable quantity of sample ground through 1 mm mesh into Erlenmeyer flask. Add 100 ml of 3N HCl and boil for 1 h. Cool at room temperature and filter through a filter paper and rinse with diluted water to remove all hydrochloric acid. Remove the moisture of the sample by drying it in an oven at 105°C for 24 h. Extract the oil/fat from the sample using a soxhlet system for 24 h. Remove the diethyl ether and replace with clean diethyl ether. Continue the extraction for another 8 h.

C-1.4.2 Evaporate all the diethyl ether extracts and recover the diethyl ether for recycling. Dry the diethyl ether extract containing fat/oil at 100°C for 1 h. Use the sample for the determination of free fatty acid.

C-2 REAGENTS

C-2.1 Ethyl Alcohol, ninety-five percent (by volume), or rectified spirit (conforming to IS 323), neutral to phenolphthalein indicator.

C-2.2 Phenolphthalein Indicator Solution — Dissolve 1 g of phenolphthalein in 100 ml of ethyl alcohol.

NOTE — When testing oils or fats which give dark coloured soap solution, the observation of the end point of the titration may be facilitated either: (a) by using thymolphthalein or alkali blue 6B in place of phenolphthalein, or (b) by adding one millilitre of a 0.1 percent (*w/v*) solution of methylene blue in water to each 100 ml of phenolphthalein indicator solution before the titration.

C-2.3 Standard Aqueous Potassium Hydroxide or Sodium Hydroxide Solution, 0.1 N or 0.5 N.

C-3 APPARATUS

C-3.1 Analytical Weighing Balance

C-3.2 Conical Flask, 250 to 300 ml capacity.

C-3.3 Water Bath

C-4 PROCEDURE

Mix the oil or melted fat thoroughly before weighing. Weigh accurately a suitable quantity of the cooled oil or fat in a 200 ml conical flask. The weight of the oil or fat taken for the test and the strength of the alkali used for the titration shall be such that the volume of alkali required for the titration does not exceed 10 ml. Add 50 to 100 ml of freshly neutralized hot ethyl alcohol, and about 1 ml of phenolphthalein indicator solution. Boil the mixture for about 5 min and titrate while as hot as possible with standard aqueous alkali solution, shaking vigorously during titration. Calculate the free fatty acid as given in **C-5**.

C-5 CALCULATION

C-5.1 Free Fatty Acids

C-5.1.1 The acidity is frequently expressed as the percentage of free fatty acids present in the sample. The percentage of free fatty acids in most of the oils and fats is calculated on the basis of oleic acid; although in coconut oil and palm kernel oil it is often calculated in terms of lauric acid, in castor oil in terms of ricinoleic acid, and in palm oil in terms of palmitic acid. The calculation of fatty acids for soyabean/soya products is as follows:

$$\text{Free fatty acids, in terms of oleic acid, percent by mass} = \frac{28.2 \times V \times N}{W}$$

where

V = volume of standard potassium hydroxide solution used, in ml;

N = normality of standard potassium hydroxide solution; and

W = weight of the material taken for the test, in g.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

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